Inter-laboratory Calibration of Redox Potential and Total Sulfide Measurements in Interfacial Marine Sediments and the Implications for Organic Enrichment Assessment

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Inter-laboratory Calibration of Redox Potential and Total Sulfide Measurements in Interfacial Marine Sediments and the Implications for Organic Enrichment Assessment

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ABSTRACT

Wildish, D.J., Akagi, H.M., Hargrave, B.T., and Strain, P.M. 2004. Inter-laboratory calibration of redox potential and total sulfide measurements in interfacial marine sediments and the implications for organic enrichment assessment. Can. Tech. Rep. Fish. Aquat. Sci. 2546: iii + 25 p.

Inter-laboratory calibration experiments with interfacial sediments from salmon farm and reference locations in the Bay of Fundy were highly variable for redox potential (Eh), but less so for total sulfide measurements. The coefficient of variability for total sulfide was ≤ 38% using a standard method (1:1 volumetric standard/sample:SAOB, sulfide anti-oxidant buffer). In agreement with published results the high variability of Eh was due to two factors: probe "poisoning" (formation of coatings of sulfide or oxide on the platinum probe surface which altered the electrical response) and an unstable electrode response in poorly poised, oxic sediments. The probe "poisoning" could not be removed by polishing or chemical treatment with aqua regia. We conclude that measurements of total sulfide, made by the standard method, can be used to indicate the organic enrichment stage of each sample. The high variability for Eh precludes its use in defining sedimentary organic enrichment stages. Eh might be used semi-quantitatively as an internal validation of total sulfide concentrations where the sediments are anoxic or hypoxic.

RÉSUMÉ

Wildish, D.J., Akagi, H.M., Hargrave, B.T., and Strain, P.M. 2004. Inter-laboratory calibration of redox potential and total sulfide measurements in interfacial marine sediments and the implications for organic enrichment assessment. Can. Tech. Rep. Fish. Aquat. Sci. 2546: iii + 25 p.

Les expériences d'étalonnage interlaboratoires sur des sédiments interfaciaux provenant de stations salmonicoles et de sites témoins dans la baie de Fundy ont donné des résultats très variables en ce qui concerne le potentiel rédox, mais moins variables dans le cas des sulfures. Le coefficient de variabilité pour les sulfures totaux était de ≤ 38 % quand on employait une méthode standard (1 : 1 étalon volumétrique/échantillon : solution tampon SAOB). Conformément aux résultats publiés, la forte variabilité du potentiel rédox était due à deux facteurs : « intoxication » de la sonde (formation de couches de sulfure ou d'oxyde sur la surface de la sonde de platine, ce qui altère la réponse électrique) et réponse instable de la sonde dans des sédiments oxiques peu équilibrés. Il a été impossible d'éliminer l'« intoxication » de la sonde par polissage ou traitement chimique à l'eau régale. Nous en concluons que les mesures des sulfures totaux, faites par la méthode standard, peuvent servir à indiquer le degré d'enrichissement organique de chaque échantillon. Par contre, la forte variabilité du potentiel rédox interdit d'utiliser cette mesure pour définir le degré d'enrichissement organique des sédiments. Le potentiel rédox pourrait toutefois servir de façon semi-quantitative pour une validation interne des concentrations de sulfures totaux quand les sédiments sont anoxiques ou hypoxiques.

INTRODUCTION

Previously we presented a rationale for environmental monitoring of sediments to detect organic enrichment based on geochemical methods which was applicable to the salmon mariculture industry in New Brunswick (Wildish et al. 1999). The measurement of redox potentials (Eh) and total sulfides ($S^{=}$) in sediment pore water has been adopted by the N.B. provincial regulating authority for the salmon culture industry (Anon. 2001) and an operating protocol has been devised (Anon. 2002).

Following the publication of Wildish et al. (1999) we received feedback from users, or potential users, of geochemical methods for monitoring salmon culture organic enrichment effects. These included Brooks, K. (pers. comm. 2001) and Brooks and Mahnken (2003), who found Eh measurements to be highly variable. Part of the reason was thought to be that the large circular cross section (typically 6.5 mm diameter) of most commercial platinum electrodes was not suitable for sampling surface layers, especially if inserted sideways into the core tube, where Eh often rapidly changed over a few millimeters depth. Soo, A. (pers. comm. 2001) questioned the chemical conditions in which total sulfide was determined by ion analysis, in particular, whether sediment matrix effects influenced the sulfide results. Because of these and other more informal queries, we have undertaken the additional work reported here to investigate the suitability of geochemical methods for compliance monitoring purposes in the Bay of Fundy mariculture industry. The central part of this work was an inter-laboratory calibration experiment involving many of the contractors and regulatory personnel involved with the Bay of Fundy mariculture industry. Subsequent specific investigations for Eh involved varying probe filling solution concentration, the causes of variability among probes and the effect of sediment water content on the determination. In addition to the inter-calibration experiment for sulfide we compared different types of sulfide probes, different ratios of SAOB (sulfide anti-oxidant buffer) to standard Na_2S solutions and the effect of seawater concentration on the determination.

As a result of the inter-laboratory calibration and further experiments reported here it became clear that redox potential could not be used as a variable to define the four stages of the organic enrichment gradient used in compliance monitoring. The reader will find the newly recommended field sampling and subsampling methods in the METHODS section. The rationale for the changes made is presented in the DISCUSSION section.

METHODS

In the earlier publication (Wildish et al. 1999) we focused on practical sampling, but here stress two goals:

- the comparison of enriched and reference locations (see Wildish et al. 2001). The comparison method can be associated with a scientific hypothesis: the null being that the two locations are similar in geochemical characteristics and the alternative hypothesis being that they have different characteristics, with respect to organic enrichment; and
- application of geochemical methods to compliance monitoring by comparing farm sites with an empirically derived organic enrichment gradient, which is based on benthic macrofaunal characteristics.

FIELD SAMPLING METHODS AT DEPTHS <30M

The diver should be positioned at the mid-cage point for sampling. This can be achieved by observing air bubbles from the submerged divers at the surface and guiding their movements by telephone contact. An approximate position at the time of sampling can be obtained by use of a handheld GPS or from the attending vessel. A 1- or 0.5-m^2 metal quadrat is laid out at the site and all replicate samples made from within it. The reference location is chosen so that it is not within the immediate zone of influence of organic enrichment, shares the same sediment and other environmental characteristics, as much as possible and is close (50-200 m) to the farm site. It too should have a 1-m^2 quadrat laid out, from which an equal number of replicate samples are taken.

The Hargrave corer for interfacial sediment sampling (Wildish et al. 2003) is deployed by divers. It is an open ended plexiglass box, 17.5 cm x 15 cm x 35 cm with a hinged lid (Fig. 1) and lower end which is cut diagonally with a sliding base to collect undisturbed sediment samples with the overlying water. The corer has stainless steel fittings and a large aluminum handle so that the diver can carry and position it in sediments (not shown in Fig. 1). After the diver pushes the corer into the sediment to about half to three quarters of its depth, the backplate is pushed closed and the hinged lid shut for transfer to the surface. It is stored in the upright position with the sediment surface uppermost, on a wooden stand. The volume capacity of the completely full corer shown in Fig. 1 is 6.2 L and the area sampled is 0.0263 m^2. The Hargrave corer can be fabricated by Plastic World and Design, Burnside, Dartmouth, Nova Scotia (Tel: 902-468-3233).

FIELD SAMPLING METHODS AT DEPTHS >30 M

We have no further suggestions to add to the earlier report. The areas and volumes (assuming a 2 cm deep surface layer) sampled for the core tubes and Hunter-Simpson grab are:

- 50 cm long x 5 cm wide tube Area $\pi r^2 = 3.14 \times 2.5^2$ cm $= 19.63$ cc
  Surface volume = Area x 2 cm = 39.25 cc
- Grab, 31 cm x 31 cm Area = 961 cm^2
  Surface volume = Area x 2 cm = 1922 cc

SUBSAMPLING

Subsamples are taken from the sediment surface (that is the top 0–2 cm from an intact sediment core). This is done by pushing a cut-off syringe into the sediment at an approximately 45^0 angle, then gently withdrawing 5 cc of sediment slurry so as to exclude air spaces. Filled syringes are "capped" with a plastic lid, which fits tightly over the barrel of the syringe (to exclude air), washed, and then placed on ice in a cooler chest. These samples can be stored in a refrigerator for a maximum of 72 h before analysis (Wildish et al. 1999). We suggest taking a minimum of five replicate samples from each core/grab sample.

To prepare cut-off syringes: obtain plastic medical syringes, e.g. Becton-Dickson 5 cc (Fisher # 14-823-35). With a sharp, sturdy knife, cut off the Luer lock tip just to the zero mark on the

barrel (thus leaving the syringe barrel open ended). After washing, syringes and caps can be re-used.

LABORATORY

The new subsampling metthod is designed to allow Eh and $S^{=}$ to be determined on the same replicate sample. After alllowing the filled syringes to reach ambient temperature, each is extruded into a 50-cc plastic beaker (Fisher catalogue # 02-591-10A) and the temperature and Eh recorded. Push the Eh probe into the sediment for a few mm and wait until the mV reading on the meter has stabilized (Eh drift < 10 mV/min) before recording it. Once this is completed, add a 5 cc volume of SAOB to begin the sulfide determination (see below). We recommend a minimum of five replicate subsamples be analyzed for both Eh and total sulfide.

Eh is measured as in Wildish et al. (1999), except that the filling solution to be used is 4 M KCl (and not 0.2 M as stated in error in Wildish et al. 1999). Regular cleaning of the platinum probe surface is required by polishing (Anon. 1983). We have also undertaken the cleaning of a black precipitate which may form on inner surfaces of the probe. To do this, a small brush is used against the surface of the ceramic reference until clean, and then flushed with de-ionized water. The probe is re-assembled and fresh filling solution added, allowing 24 h for the probe to equilibrate. We have also used a more drastic treatment to recondition the probe (Nordstrom and Wilde 1998), which involves immersing the tip of the probe in aqua regia for <1 min, followed by flushing with deionized water. Aqua regia is carefully prepared by mixing 1 vol. concentrated nitric acid with 3 vol. concentrated hydrochloric acid in a fume hood. For short-term storage (<3 wk) redox potential probes are stored in beakers containing de-ionized water. For longer periods when not in use the filling solution is drained, the probes flushed clean with deionized water and stored dry. The results are expressed normal to the hydrogen electrode by the equation in Wildish et al. (1999). If solution temperatures are recorded, use equation (1) or (2) shown below. Instructions were given earlier for the Accumet ion meter which will not apply to other makes. Readers should follow the maker's instructions for their ion meter in setting up and making determinations.

Sulfide determinations are made as described in Wildish et al. (1999) using at least three points to establish the calibration curve from which unknown samples can be determined. We recommend the use of 0.1 M sodium sulfide as a standard for this purpose (to make a concentration range of 100, 1000, 10,000 µM). Readers with ion meters other than Accumet should follow the maker's instructions for their use. The practical lower detection limits of the Orion sulfide electrodes that we have used are ~50 µM total sulfide. If field samples <100 µM need to be accurately determined, then the results should be calibrated with Na_2S standards in the 50–100 µM range.

STATISTICAL ANALYSIS

The null hypothesis tested is generally that two independent, randomly drawn groups of samples are from the same population. Because of the known contagious distribution of geochemical variables in sediments at decimeter scales (Wildish, unpublished), non-parametric tests are recommended. We have used the non-parametric equivalent of the t-test, that is the Mann–

Whitney U-test, to determine between the null and alternative hypotheses. It is a test of the rank order between median values of farm and reference samples. The computations involved are shown in Elliot (1979) and if n_1 or n_2 is >20 the normal deviate can be used to calculate the U statistic. Where n_1 or n_2 is <20 the normal deviate cannot be used and individual U_1 and U_2 values must be calculated. Table 14 in Elliot (1979) gives tests of significance at the 5% level for sample sizes <20. The computations can be completed in MS Excel, although this software does not specifically include this test. Student t-tests were used for probe comparisons.

RESULTS

REDOX POTENTIAL

Probe filling solution (KCl) concentration

The internal filling solution for the redox potential ion probe is supplied as KCl of stated molarity, saturated with AgCl (e.g. Orion # 900011 is 4.0 M KCl). We diluted the latter solution with deionized water in the following experiments. This resulted in a precipitate which was removed by centrifuging. We tested three ion probes (DW #1, 2 and 3) all of which were Orion model 96-78 platinum redox electrodes, against freshly prepared Zobell's A and B solutions. The results are shown in Table 1. The Orion platinum redox electrode instruction manual (Anon. 1998) states that solution B should be ~66 mV greater than solution A. The mean and standard deviation, to the nearest millivolt at all molarities tested in Table 1 is: DW 1 = 66 ±4, DW 2 = 65 ±4, DW 3 = 66 ±4 mV. These values are within the specifications suggested by Orion (Anon. 1998).

Table 1. Redox potential as Eh, mV (unconverted) for the same batch of Zobell's solutions A and B. The probes are filled with different molarities of KCl. "Diff" is the difference between B and A readings. The probe time indicates the period for equilibration after changing the filling solution.

KCl molarity	Temp. ^{0}C	Probe time, h	DW 1			DW 2			DW 3		
			A	B	Diff	A	B	Diff	A	B	Diff
0.2	24.7	24	189	260	71	182	251	69	151	222	71
0.2	22.0	18	199	267	68	195	259	64	153	221	68
0.2	23.0	6	194	265	71	189	260	71	146	218	72
0.5	20.1	17	224	290	66	220	284	64	181	248	67
1.0	21.0	6	228	294	66	219	287	68	193	258	65
2.0	21.5	2	234	298	64	233	296	63	209	274	65
4.0	19.8	17	244	305	61	242	304	62	239	300	61
4.0	20.6	2	239	302	63	242	303	61	237	300	63
4.0	20.6	1	238	301	63	241	303	62	238	300	62

The values for 4.0 M KCl in Table 1 can be converted to Eh_{NHE} by reference to Table 1 in Wildish et al. (1999), or for convenience we have expressed the data there as an equation:

$$Eh_{NHE}\ mV = 224 - (\text{temperature, } ^{o}C) \quad (1)$$

For other molarities based on data in Table 1 (Wildish et al. 1999) the following multiple regression equation can be used:

$$Eh_{NHE}\ mV = 285.4 - 16.23\ (\text{molarity}) - 0.825\ (\text{temperature, } ^{0}C) \quad (2)$$

Inter-laboratory calibration experiment

The aim was to determine the variability introduced by different ion probes and meters (operated by six groups of analysts) when measuring Eh and total sulfide in the same sediment samples. Hargrave cores, obtained by hard hat diving from the mid-point of net pens in Lime Kiln Bay on the 7th August, 2003, were taken from five different pens. Cores were also taken at a reference location ~50m away from the pens, with sampling in an area of a few square meters, to yield a total of five reference cores. Precise sampling locations were recorded with the aid of GAPS technology (McKeown et al., in prep.) and are given in Appendix 1. All 10 cores were transported to the lab for subsampling as described in the METHODS section. Subsampling was completed by others, so that each analyst was provided with five replicated, refrigerated, syringe subsamples from the farm (F) and five reference (R) subsamples. Zobell's A and B solutions were all pre-prepared as a single batch and provided to each analyst with a mercury bulb thermometer to record reaction temperatures. Six groups of analysts took part; they are not identified, but a complete list of persons involved is given alphabetically in the Acknowledgements section. Each analyst supplied his/her own ion meter and probes. These included the following meters: Orion (=ThermoOrion) SA 720, 290A+, Fisher - Accumet AP25, AP63, and 1003, and Hanna Instruments HI 9025. Redox potentials in Zobell solutions at the beginning, during and at the end of the inter-laboratory experiment for each analyst are given in Appendix 2. All of the Zobell results are within the expected range for acceptable probe responses by this test. Probes used (the figure in brackets indicates the probe in Table 2) included: Cole Parmer ORP Redox/pH electrode (1), Orion 96-78 (2), Orion 96-78 (3), Hanna HI 3230 (4), Orion 96-78 (5) and Hanna HI 3230 (6). All used 4 M KCl filling solution.

Results are shown in Table 2. The median for six probes, each with five replicates is for Farm -161 mV and for Reference 69 mV. The Mann-Whitney U-test shows that H_0 can be rejected ($U_1=0$, $U_2=36$, $p<0.05$) and the H_1 accepted, that the farm and reference locations differ in Eh_{NHE}. Two other pertinent results from Table 2 are that:

- probes 3 and 4 (Table 2), which were recently purchased and used for the first time during the inter-laboratory calibration experiment ("new" probes), gave significantly higher values in reference sediments than "old probes". This included all of the other probes, which had been used prior to the inter-laboratory experiment, often exhaustively, in both anoxic and oxic sediments. The differences were >200 mV ("new" probes 3 and 4 = 189±24.7 and "old" probes 1, 2, 5 and 6 = -24±49.0 mV; mean ± standard deviation); and

- the farm replicates showed less variability (S.D. ranging from 3–32 mV) than the reference replicates (S.D. from 14–71 mV).

Table 2. Inter-laboratory calibration experiment. Eh_{NHE} mV from Lime Kiln Bay on 7th August, 2003, as determined by six independent analysts/probes. Five replicated determinations were made on the two, mixed farm and reference sediments taken from the surface 0–2cm depth. Sediment water content (mean ± S.D., N = 3) for farm = 70.7 ±1.97 and reference = 63.5 ± 0.75. See Appendix 2 for Zobell values recorded during this experiment.

Farm	Probe					
Replicate	#1	#2	#3	#4	#5	#6
1	-156	-150	-153	-91	-274	-161
2	-153	-176	-161	-99	-236	-196
3	-147	-181	-164	-98	-206	-206
4	-154	-170	-165	-97	-203	-196
5	-163	-171	-170	-95	-198	-211
Mean	-155	-170	-163	-96	-223	-194
Std.Dev	6	12	6	3	32	20
Std.Err	3	5	3	1	14	9
Reference Replicate						
1	42	32	177	245	-70	52
2	-6	14	181	197	-41	-46
3	-14	61	185	203	-61	-110
4	17	18	159	200	-81	-112
5	-4	17	154	186	-80	-107
Mean	7	28	171	206	-67	-65
Std.Dev	23	20	14	23	16	71
Std.Err	10	9	6	10	7	32

To determine if the approximately 3 h that cores were in transport to the lab affected the results, Eh and temperature were measured as soon as the cores were brought on deck. This was done by inserting the probe vertically into the top few millimeters of the sediment interface. Results with two probes (both Orion 96-78), DW 2 and DW 3 (probe # 3 in the inter-calibration experiment) gave the following results as Eh_{NHE} for the mean ± standard deviation: DW 2 ("old" probe which was not used in Table 3), Farm = -117± 28 ,Reference = -15 ± 11 mV; DW 3 ("new" probe as #3 in Table 3), Farm = -97 ± 38, Reference = 142 ± 22 mV (see Appendix 3). After storing for 24 h in the refrigerator subsamples (N = 5) from the intercalibration experiment were re-tested with BH 5 ("old" probe # 5 in the inter-calibration experiment) with the result: BH 5, Farm = -206 ±6, Reference = - 48 ± 5 mV (Appendix 3). Despite differences in preparation of the sediment the results are not significantly different from the mean for the same probes in Table 2, for both immediate sampling and storage experiments.

Table 3 Eh_{NHE} mV at 23^0C for Lime Kiln Bay intertidal sediments with untreated and physically cleaned probes. The mean water content of the sediment was 46.10%. N = 5. See Appendix 4 for the original data

Treatment	Statistic	DW 2 - old	DW 3 - new	BH 5 - old
Untreated	Mean	174	274	127
	S.D.	33	24	34
	S.E.	15	11	15
Probe polishing	Mean	200	301	135
	S.D.	38	28	10
	S.E.	17	13	5

Causality of differences among probes in the inter-laboratory calibration experiment

We tested the working hypothesis that "old" probes had been poisoned to a variable degree by the deposition of oxides or sulfides on the platinum surfaces (see DISCUSSION for references). The poisoning effect could cause a slowing of the electromotive response and thus reduce the mV reading during the limited measurement times that were used. The tests involved using the cleaning procedures outlined in the METHODS section as well as using a different filling solution. We had two probes available from the inter-laboratory experiment: #3 (= DW 3 "new") and #5 (=BH 5 "old"). In addition, we used two "old" probes: DW 1 and DW 2 and purchased a new one (DW 4).

We obtained oxic, interface, silt/clay/sand sediments (top 2 cm scraped into a bucket) from the intertidal zone of Lime Kiln Bay on 25th August, 2003. The oxic layer above the RPD at the sampling site was deeper than 5 cm. On return to the laboratory the sediment was gently mixed to a smooth consistency and subsampled into replicated 5-cc cut-off syringes which were sealed by capping. These subsamples were refrigerated and analyzed after the syringe samples were equilibrated to lab temperature 48 h later. A test was made to determine if conventional polishing of the platinum tip removed the poisoning effect. After recording the redox potential of oxic sediments with each of the untreated probes (Table 3), each was disassembled, drained, cleaned with deionized water, and the platinum electrode surface polished before re-filling with 4 M KCl. Redox potential measurements with the cleaned probes are shown in Table 3. The results do not support the view that cleaning had any significant effect on the response of any of the 3 probes. All of the probes were checked against Zobell's A and B solution during these tests and gave potential differences, B-A, of 62-69 mV.

The "new" probe in Table 3 consistently gave significantly higher readings than the "old" probes, both before and after probe polishing and changing the filling solution.

Following communication with Orion, we tested a new filling solution. It was prepared by adding KNO_3 (10% weight/volume) to deionized water and then adding enough KCl to make a 10^{-3} M solution. Prior to adding the new filling solution in the second part of the experiment, we

also disassembled the old probes and used a fine brush to clean a black deposit from the inner probe surfaces. The results are shown in Table 4. As found earlier, the Zobell B–A differences were satisfactory with 4 M KCl as the filling solution. For oxic sediments the new probes responded with Eh values which were >100 mV higher than for "old" probes. In sulfide-amended sediments this difference disappeared as expected (due to better poising in sulfide rich sediments, see DISCUSSION). Results with the KNO_3/KCl filling solution show that the Zobell B–A differences are also satisfactory (63 mV), although the numbers are an average of 14 (range 2–29) mV higher. For the oxic sediment test with the new filling solution the difference between old and new probes had risen to >200 mV.

Table 4. Comparison of four Orion 96-78 probes with different filling solutions. Top half of table filling solution (KCl), bottom half with (KNO_3/KCl). "Old" probes reconditioned for the bottom half of the table. Units are uncorrected Eh mV at a temperature of 25^0C (where C = +199 mV). The standard solutions results are single readings and the sediment readings a mean of three determinations (see Appendix 5).

		"Old" Probes		"New" Probes	
Solution	Treatment	DW # 1	DW # 2	DW # 3	DW # 4
FILLING SOLUTION (4M KCl)					
Zobell's A		204	222	214	231
Zobell's B		279	289	287	296
Orion Standard		211	208	210	214
Sediment	Mixed	-322	-348	-208	-239
Sediment	Add Na_2S*	-500	-445	-512	-514
Zobell A		228	224	220	232
Zobell B		292	286	290	296
Orion Standard		210	210	210	215
FILLING SOLUTION (KNO_3/KCl)					
Zobell A		233	233	229	233
Zobell B		296	296	293	296
Orion Standard		217	217	217	219
Sediment	Mixed	-63	-65	248	215
Sediment	Add Na_2S*	-372	-299	-182	-195
Zobell A		227	230	226	229
Zobell B		293	294	291	292
Orion Standard		217	218	217	219

*10 mL 0.3 M $Na_2S.9H_20$

We also tested chemical cleaning with aqua regia (Nordstrom and Wilde 1998). The sediments used were oxic, sandy/silt particles scraped from the surface of intertidal deposits at Pottery Creek, collected just before use and well mixed with a glass spatula. The Eh readings shown in Appendix 6 were made by placing the probes in Zobell's solution A, then B, then sediment, followed by repeats in the same sequence until 10 readings of each solution and sediment had

been obtained. After each reading, the probes were rinsed with deionized water and dried with Kimwipes. All four of the probes used gave results with Zobell's solutions A and B which are within the Orion specifications (A = 234±9 mV and B = 300±9 mV), both before and after the aqua regia treatment. The old probes again gave lower readings in oxic sediment than the newer ones (Table 5) both in the "before" and "after" parts of this test. In the "before" part of the test for sediments each probe gave different readings and this persisted into the "after" part of the test. In oxic sediments probe # 1 became more negative, # 2 less negative, #3 was unchanged and # 4 became more negative after acid treatment.

Table 5. Comparison of four Orion 96–78 probes with 4 M KCl filling solution and before/after treatment for 1 min with aqua regia (1 vol conc. HNO_3 : 3 vol conc.HCl). Units are uncorrected Eh mV (to correct to $_{NHE}$ at 25^0C use c = 199 mV). All readings are means corrected to the nearest mV (± standard error) of the 10 observations shown in Appendix 6.

	"Old" Probes		"New" Probes	
Solution/Treatment	DW # 1	DW # 2	DW # 3	DW # 4
		BEFORE		
Zobell A	241(1.67)	231(0.52)	233(1.07)	238(1.86)
Zobell B	305(1.37)	298(0.46)	300(0.87)	306(1.88)
Sediment, mixed	-35(31.05)	-257(14.13)	235(5.27)	171(18.15)
		AFTER		
Zobell A	232(0.82)	237(0.42)	235(0.31)	233(0.52)
Zobell B	297(0.54)	299(0.390	299(0.19)	300(0.30)
Sediment, mixed	-341(9.79)	-129(12.5)	227(7.06)	9(21.0)

Effect of water content in oxic sediments

The aim was to test the hypothesis that the lower water content of reference sediments, such as those from the Lime Kiln Bay intertidal (see also Table 2) affected Eh measurement and its variability. The probe filling solutions were 4 M KCl and these experiments were run prior to the tests shown in Tables 4 and 5. We changed the water content of the mixed sediment by adding locally available seawater (PSU = 30 ppt) in batches of 50 mL, followed by slow stirring. We tested three probes as is shown in Table 6. The raw data on which the means are calculated is shown in Appendix 4.

Again mV values measured with "new" probes were higher than for the "old" probes. T tests for difference between the means show that within each probe increasing water content had no effect on the Eh. This is shown graphically in Fig. 2 and confirms that there is little effect of water content for DW 2, BH 5 and for DW 3.

Table 6. Eh_{NHE}, mV at 23^0 C for Lime Kiln Bay intertidal sediments (interfacial sample). Means are based on N = 5. Percentage water content is varied by addition of seawater to the sediment. Original data is in Appendix 4.

Statistic	Probe			Percent water content
	DW 2 old	DW 3 new	BH 5 old	
Mean	174	274	127	46.10
SD	33	24	34	0.27
SE	15	11	15	0.12
Mean	217	361	103	49.78
SD	35	3	44	0.28
SE	16	1	20	0.12
Mean	202	366	148	55.69
SD	36	5	61	0.35
SE	16	2	27	0.16
Mean	139	380	183	60.86
SD	34	28	62	0.06
SE	15	13	28	0.03

Recognition of "poisoned" probes

One way to determine the functional status of a probe platinum surface is to record potentials over time when continuously immersed in oxic seawater. Typical results of this treatment are shown in Table 7. The results suggest a probe-specific time to reach an equilibrium, with a clear influence of Zobell's solution on the observed potential. From Table 7 the two BH probes are "old" and the two DW probes are "new".

Table 7. Eh as unconverted mV in aerated seawater (PSU = 30 ‰).

Elapsed time, h	Temp. ^{0}C	BH 3	BH 5	DW 3	DW 4
0*	11.7	59	31	296	250
1	15.5	46	-128	297	236
2	19.0	43	-128	284	239
11	22.4	-27	-131	185	209
24	19.2	-55	-121	156	208
44	20.3	-115	-124	151	201
66	21.8	-111	-122	107	212
67*	21.9	74	29	256	264
69	21.7	36	-117	72	216
70*	22.9	74	32	226	248

*All probes immersed in Zobell's solution prior to this measurement.

TOTAL SULFIDE CONTENT OF SEDIMENT PORE WATER

Comparison of different ion probes

Accumet ion meters (Fisher model AP25, see Anon. 1997) were used as previously (Wildish et al. 1999). The following ion probes were tested:

1. Orion #9616 combination silver/sulfide electrode with Orion # 900061 or 900062 filling solutions.
2. Orion #9416 silver/sulfide half cell and Orion # 90-02 double junction reference electrodes. The latter is filled with Orion # 900002 (AgCl) in the inner, and Orion # 900003 (KNO_3) in the outer chamber. This was the probe used in Table 10 probe comparison tests.
3. Orion # 9416 silver/sulfide half cell with Orion # 90-01 single junction reference electrode filled with Orion #900001 solution (contains Na, K, NO_3, Cl ions saturated with Ag). This probe was not used in the Table 10 calibration tests.
4. Thomas combination electrode (gel, does not require filling solution)
5. DetectIon combination electrode (gel, does not require filling solution)

Probes were compared by measuring the electrode responses in the same freshly prepared solution of $Na_2S.9H_2O$ (1000 µM). The sulfide solution was prepared with de-ionized water through which nitrogen gas in a fine stream was bubbled for 10 min (degassing). The determinations were made by mixing SAOB and standard in a 1:1 ratio. The results are shown in Fig. 3 and suggest that all four probes tested have a similar slope response to sulfide, although intercept values are different. Thus at 1000 µM total sulfide, there is a spread of ~70 mV with DetectIon showing the greatest and Orion # 9616 the least. Reproducibility of the measure seems to be higher for the liquid filled Orion probes (Table 8) and, consequently, we have not pursued the gel-filled probes further.

Table 8. mV results for three concentrations of sulfide standard. The second set of measurements was approximately 15 min after the first.

Concentration µM	Probe							
	Orion # 9616		Thomas		Orion # 9416		DetectIon	
	First	Second	First	Second	First	Second	First	Second
100	741	740	749	772	774	775	808	827
1000	774	773	789	807	808	807	843	858
10000	805	804	821	838	840	839	876	888

Effect of Varying Ratio of SAOB: standard

All determinations of sulfide for testing the effect of varying the ratio of SAOB to standard were made with an Orion # 9616 probe. A new stock of sodium sulfide standard (1000 µM) was made in degassed de-ionized water. A determination of total sulfide was then made in the standard way

for a 1:1 volume ratio of SAOB: standard. More SAOB was then added to the beaker so that the ratios tested were 3:1, 7:1, 11:1, 15:1 and 19:1. This took approximately one-half hour, after which another 1:1 SAOB:fresh standard was measured. We have accounted for the dilution by the serial addition of SAOB. The results are shown in Fig. 4 and Appendix 7 and suggest that as more SAOB is used with a fixed volume (5 mL) of sample, more sulfide is extracted, with the process becoming asymptotic at >7:1.

Effect of varying salinity

We determined the effect of possible carryover of seawater in the sediment sample by replacing dilution water (deionized water) by seawater (locally available, filtered, ~30 PSU) as shown in Table 9. The results show that there is no effect of salinity on this determination

Table 9. Effect of seawater concentration at a 1:1 ratio of SAOB: standard (10,000 µM $Na_2S.9H_20$) on measurement by ion analysis of total sulfide.

SAOB, mL	Seawater, mL	$Na_2S\ 9H_20$ (10,000 µM), mL	Deionized water, mL	Total sulphide, µM
10	9.0	1	0.0	1030
10	7.5	1	1.5	1140
10	5.0	1	4.0	1220
10	2.5	1	6.5	1200
10	1.0	1	8.0	1200
10	0.0	1	9.0	1000

Inter-laboratory calibration experiment

All of the probes used in this experiment were either Orion # 9616 combination electrodes (with either filling solution A or B), or Orion # 9414 with a double junction reference electrode (Orion # 90-02 with inner filling solution of AgCl, Orion # 900002, and outer filling solution of KNO_3, Orion # 900003).Probe # 6 results were omitted from Table 10 below because it transpired that they were made without adding SAOB to the sediment samples.

For the remainder of the samples, N = 25, the median is 3266 for the farm and 705 µM for the reference station. A Mann-Whitney U test showed that total sulfides in farm sediments are significantly greater ($U_1 = 0$, $U_2 = 625$ $p < 0.001$) and the H_0 that the two sets of samples are from the same population, rejected. The overall coefficient of variation for this data is 38% for farm and 32% for reference samples.

Checking the sulfide standard concentrations

A potential problem with sulfide standards is that they may oxidize in air. This instability of sulfide standards is more pronounced the more dilute the concentration becomes. We

Table 10. Inter-laboratory calibration experiment. Total sulfide in sediment pore water as μM, from Lime Kiln Bay as determined by six independent analysts/probes (#6 was omitted because of a mistake during analysis). Five replicated determinations were made on farm and reference sediments.

Farm	Probe				
Replicate	#1	#2	#3	#4	#5
1	3000	3266	6150	6320	2950
2	2220	2504	6170	3420	3020
3	2020	3266	6180	3510	2530
4	3000	3490	4650	4110	2500
5	2180	2860	5240	3500	2440
Mean	2484	3077	5678	4172	2688
SD	477	393	701	1232	274
SE	213	176	313	551	123
Reference Replicate					
1	318	925	734	705	488
2	373	709	560	516	1030
3	568	621	754	713	773
4	445	758	1130	563	720
5	469	709	1240	629	630
Mean	435	744	884	625	728
SD	95	112	288	86	200
SE	43	50	129	39	90

independently check the concentration of sulfide standards by titration with Pb $(ClO_4)_2.6H_20$ (Orion 948206; Fisher # C13 641 773). The reactants are:

25 mL 0.03M $Na_2S.9H_20$
25 mL SAOB
Lead standard in the burette (Metrohm 645 Multi-Dosimat – Auto-buret)

Near the sharp end-point of the titration the lead standard is added in 0.1-mL increments. Calculations are as follows:

Volume Pb standard added = V_{std} mL
Volume sulfide standard added = V_S mL
Concentration of Pb standard = C_{std} M

Then concentration of standard sulfide = $(V_{std}/ V_S)*C_{std}$

DISCUSSION

REDOX POTENTIAL MEASUREMENTS

According to Schulz (2000), unexplainable fluctuations of Eh ± 50 mV can be expected when measuring reduction potentials in sediment with platinum electrodes. Schulz's results were

obtained with well maintained and polished electrodes and use of a glove box. Thus, sediments were examined in an inert atmosphere (nitrogen or argon) free of oxygen. All of the results presented in our study were made in air without a glove box. During the inter-laboratory calibration experiment (Table 2) two probes (# 3 and # 4) consistently gave higher Eh potentials (>100 mV) in reference sediments. Both probes were recently purchased (Orion 96-78 and Hanna Instruments HI 3230). Further testing showed that "new" and "old" probes behaved differently. Cleaning "old" probes by polishing resulted in small (<100 mV) temporary increase in potential, although as soon as they were placed in sediments with a high sulfide content the potentials rapidly became negative again. Doyle (1968) considered the performance of platinum redox surfaces in iron-containing freshwaters and described the effect of oxide coatings which influenced potentials. Whitfield (1974) and Enriquez et al. (2001) mention the formation of oxides on probes in marine, oxic sediments and sulfides in anoxic conditions, which influences the performance of these probes. Thus "old" probes which have repeatedly been exposed to oxic and anoxic sediments will become "poisoned" in this way and respond more negatively than "new" ones, particularly in poorly poised sediments. During this study we sought ways to remove the poisoning effect and found that physical (polishing) and chemical (with aqua regia) treatment did not alter this effect in old probes. Internal cleaning and use of a different filling solution (KNO_3/KCl) also failed to rejuvenate poisoned probes. The use of standard solutions, e.g. Zobell's, failed to detect the poisoning effect because in well poised solutions (such as Zobell's and anoxic sediments where the $HS^-/SO_4^=$ couple is dominant) the response is rapid, because of the single redox couple present. In poorly poised oxic surface sediments, where macrofaunal bioturbation has carried dissolved oxygen to a few centimeters depth, the probe poisoning effect becomes noticeable. Thus standard solutions are only useful in detecting physical or electrical damage to the probe and not to probe poisoning. Probe poisoning effects could be recognized by observing potentials after immersion in aerated seawater. According to Whitfield (1974), oxide coated platinum surfaces are acting as oxide electrodes in oxic sediments and are actually measuring pH.

Another finding from the inter-laboratory calibration experiment (Table 2) was that variation within each probe replicate was greater from sediment reference samples (SD = 14-71 mV) than for farm samples (SD = 3-32 mV). This is consistent with the interpretation that oxic sediments, above the redox potential continuity, are poorly poised (Sigg 2000). Possible multiple redox potential couples are present including O_2/H_20, $NH_4^+NO_3^-$, $NH_4^+/N_{2\,(aq)}$, $HS^-/SO_4^=$, Fe^{3+}/Fe^{2+}, $CH_{4(aq)}/HCO_3$ (Sigg 2000). The oxic sediment is not at equilibrium because of the many redox couples present and electrochemical changes do not react fast enough to yield a stable redox potential endpoint. By contrast, highly anoxic sediments, that is, those below the redox potential discontinuity (RPD), where dissolved oxygen is absent (or nearly so) are better poised because of the dominance of the HS^-/SO_4^{2-} couple.

Although it is true that commercially available redox potential probes cannot resolve the sometimes mm scale changes of Eh profile in surface sediments, this will usually not be a problem of such magnitude as probe poisoning. One obvious solution to this smearing effect is to physically homogenize the surface 0–2 cm sediment, as was done in the inter-calibration experiment.

TOTAL SULFIDE MEASUREMENTS

The evidence from the inter-laboratory calibration experiment suggests that reproducibility of the method is sufficiently good for it to be used to indicate the stages of the organic enrichment gradient shown in Tables 11 and 12. The coefficient of variability for total sulfide measurements in the inter-laboratory calibration was <38%. We have also shown that some of the sediment matrix effects, e.g., by seawater carryover, do not affect the total sulfide measurement. Whether ions from a wider range of sediment types would affect this measurement remains to be determined. We have also shown previously that sediment samples in capped, cut-off syringes, as described in the METHODS section can be stored in a refrigerator for 72 h, without change of total sulfide concentration (Wildish et al. 1999, see Table 4). We have also shown that by varying the volumetric proportions of SAOB: sample, more total sulfide can be extracted from the sample. We recommend; however, that the volumetric ratio used remains at 1:1, to maintain comparability with older data.

GEOCHEMICAL METHODS APPLIED TO COMPLIANCE MONITORING

Despite the problems identified with redox potential, both Eh and total sulfide measurements show that there are significant differences between the farm and reference sediments examined during the inter-laboratory calibration experiment. A negative Eh and high sulfide value in the farm sediment confirms that organic enrichment is present.

Compliance monitoring for the Bay of Fundy salmon culture industry (Anon. 2001; Anon. 2002) is not based on comparison of treated versus reference locations, but by comparing measurements of Eh and $S^{=}$ in sediments with ranges of values for each variable, empirically derived from observations in the Western Isles region of the Bay of Fundy (Tables 11 and 12). Individual farm sites are rated independently using Eh and total sulfide (E. Parker, pers. commun.) and the criteria listed in Table 11. In the case of a disagreement in designating the organic enrichment grouping between Eh and total sulfide, the less enriched group is the one selected.

Table 11. Correspondence between Eh_{NHE} and total sulfide values for the four classically defined organic enrichment stages (Wildish et al. 2001).

Organic enrichment stage	Eh_{NHE}, mV	Total Sulfide, µM
A Normal	>+100	<300
B+ Oxic	0–100	300–1300
B- Hypoxic	-100–0	1300–6000
C Anoxic	<-100	>6000

The inter-laboratory calibration results obtained are expressed as organic enrichment index groupings in Table 12. Consistency among the measured geochemical variables is greatest for total sulfide (all the same) and least for redox potential (Farm: 4 C's, 1 B-; Reference: 2 A's, 2 B's and 2 B-). It is clear from Table 12 that redox potential cannot be used as a reliable and

reproducible indicator of the organic enrichment stage of a given sediment sample, particularly in reference sediments where oxic conditions prevail. $S^{=}$ on the other hand provided a consistent measure of enrichment status. Hargrave et al. (1997) also found that $S^{=}$ was the single most sensitive variable for detecting differences between sediments at salmon farms and reference locations. Although the causes of Eh variability are reported in the literature (probe poisoning and poor probe performance in poorly poised sediments) and confirmed here, we did not find a way to overcome either problem. Consequently, we recommend that redox potential not be used as an indicator of organic enrichment stage. Redox potential measurements may still prove to be useful in well poised sediments, e.g. those where interface potentials are in the B- and C range. Eh can be used as a check on total sulfide concentration (there should be an inverse relationship between Eh on log. total sulfide). Total sulfide should be the only variable used to define the organic enrichment stage.

Table 12. Inter-laboratory calibration mean values of geochemical variables expressed as the equivalent organic enrichment group (see Tables 2, 10, and 11).

Geochemical variable	Analyst/Probe					
	#1	#2	#3	#4	#5	#6
Farm						
Eh_{NHE}	C	C	C	B-	C	C
$S^{=}$,µM	B-	B-	B-	B-	B-	
Reference						
Eh_{NHE}	B+	B+	A	A	B-	B-
$S^{=}$,µM	B+	B+	B+	B+	B+	

FURTHER RESEARCH

Our results for Bay of Fundy sediments show that total sulfide measurements can be used quantitatively to define organic enrichment endpoints. Additional research is needed to see whether the enrichment groups defined by $S^{=}$ measurements in the local area apply over a wider range of sediment types. In sediments from other locations, there is the possibility that other ions present might interfere with $S^{=}$ determinations made by electrochemical analysis.

Although "poisoned" redox potential probes can be recognized comparatively by immersion and monitoring their performance in aerated seawater, it is not known how to re-condition them. If the process of poisoning proves to be irreversible, a method of recognizing when the probe reaches the irreversible point would be of value in deciding when to discard it. It is clear from the literature and our own results that poorly poised, oxic sediment presents an inherent problem in compliance monitoring with redox potential measurements using ion analytical methods.

Research should also be undertaken to replace the redox potential method in defining the organic enrichment gradient, so that an independent approach is available to support total sulfide measurements in interfacial sediments. Candidate methods include; pH in interfacial sediments, sediment profile imaging (Wildish et al. 2003) and benthic macrofaunal community structure (Wildish et al. 2001).

Finally, the value of inter-laboratory experiments in detecting problems which could affect a particular methods ability to define the stages of the organic enrichment gradient are demonstrated in the work reported here. In future we recommend that all new candidate methods undergo an inter-laboratory calibration test similar to the one reported here.

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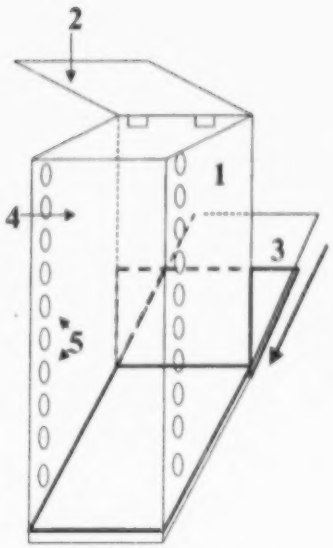

1 - open-ended plexiglass box (17.5 cm x 15 cm x 35 cm)

2 - hinged top lid

3 - bottom cut diagonally (45 °) with sliding base

4 - clear front face for photography (SPI)

5 - pre-drilled holes for Eh and sample removal

Fig. 1. Hargrave corer diagram.

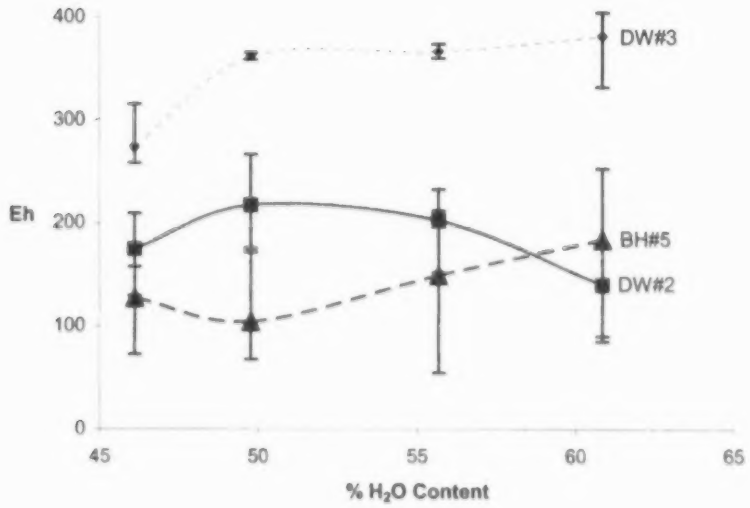

Fig. 2. Effect of sediment water content on observed Eh by three different probes.

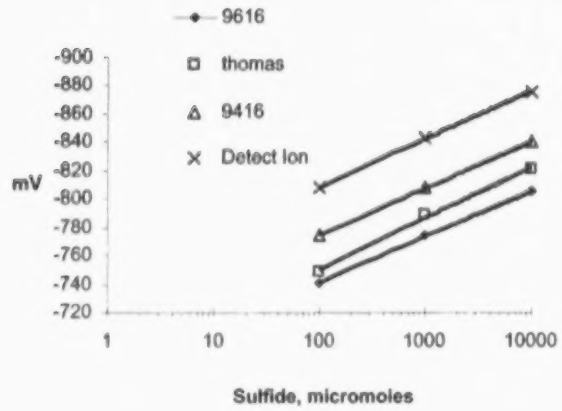

Fig. 3. Measurement of total sulfide in standard solutions by four types of sulfide probe. The volumetric ratios present were 1:1 SAOB:standard.

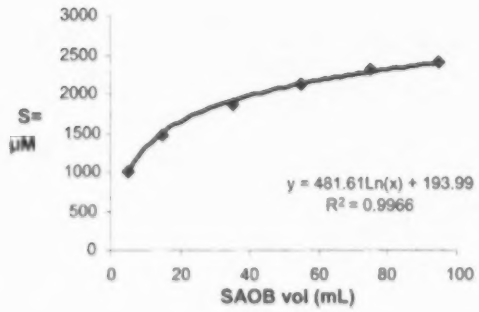

Fig. 4. The effect of the ratio of SAOB:standard.

Appendix 1. GAPS precisely determined locations sampled on 7th August, 2003.

Location	Year	Day	Time	Lat.		Long.	
Farm1	2003	219	132143	4503.719	N	6649.937	W
Farm2	2003	219	132318	4503.722	N	6649.938	W
Farm3	2003	219	132920	4503.719	N	6649.935	W
Farm4	2003	219	133127	4503.718	N	6649.935	W
Farm5	2003	219	133525	4503.719	N	6649.935	W
Ref1	2003	219	144212	4503.884	N	6649.938	W
Ref2	2003	219	143555	4503.886	N	6649.938	W
Ref3	2003	219	144243	4503.719	N	6649.934	W
Ref4	2003	219	144313	4503.885	N	6649.940	W
Ref5	2003	219	144344	4503.887	N	6649.942	W

Appendix 2. Redox potential as Eh, mv, (uncorrected) for the same batch of Zobell A and B solutions. From inter-laboratory comparison of 7th August, 2003.

Probe		Initial			Second			Final		
group	T (°c)	A	B	Diff	A	B	Diff	A	B	Diff
1	20-25	247	309	62	238	302	64	231	296	65
2	25-26	230	296	66	-	-	-	232	296	64
3	16-25	245	310	65	-	-	-	230	295	65
4	-	-	-	-	-	-	-	-	-	-
5	20-24	242	304	62	230	294	64	230	296	66
6	24-25	231	299	68	230	298	68	230	297	67

Appendix 3. E_{nhe} from Lime Kiln Bay samples (August 7th, 2003). Stored refrigerated for 24 h in a syringe (BH 5), or analyzed immediately in the field using two different probes (DW2 and DW3).

Sample #	BH5	DW2	DW3
F1	-213	-10	51
F2	-191	-127	-127
F3	-223	-157	-156
F4	-206	-123	-117
F5	-198	-169	-135
Mean=	-206.2	-117.20	-96.80
Std.Dev=	12.52	63.03	83.86
Std.Err.=	5.60	28.19	37.50
R1	-64	-42	136
R2	-36	-34	104
R3	-38	-19	155
R4	-56	21	218
R5	-45	0	97
Mean=	-47.8	-14.80	142.00
Std.Dev=	11.97	25.63	48.61
Std.Err.=	5.35	11.46	21.74

Appendix 4. Laboratory recorded Eh_{NHE} (+199 @ 23°C) for Lime Kiln Bay intertidal sediments collected on 25th August, 2003. Measurements made on samples stored in capped syringes in a refrigerator for 48 h. The last series was completed after each of the platinum probe surfaces was polished.

TTL seawater added (mL)		Probe # DW#2	DW#3	BH#5	Percent water content
0		129	315	125	46.47
		153	261	151	45.90
		183	260	157	46.28
		197	258	128	45.81
		209	274	72	46.05
	Avg	174.20	273.60	126.60	46.10
	SD	32.79	23.99	33.56	0.27
	SE	14.66	10.73	15.01	0.12
50		199	363	67	50.06
		237	358	76	49.98
		175	359	77	49.54
		207	362	171	49.43
		266	365	126	49.86
	Avg	216.8	361.4	103.4	49.78
	SD	35.32	2.88	44.31	0.28
	SE	15.79	1.29	19.82	0.12
100		224	373	164	56.09
		225	359	54	55.66
		232	366	125	55.40
		176	363	187	55.98
		151	368	211	55.29
	Avg	201.6	365.8	148.2	55.69
	SD	36.02	5.26	61.46	0.35
	SE	16.11	2.35	27.49	0.16
150		135	331	174	60.95
		165	387	84	60.81
		130	390	209	60.85
		176	403	252	60.81
		89	391	195	60.90
	Avg	139	380.4	182.8	60.86
	SD	34.07	28.28	62.17	0.06
	SE	15.23	12.65	27.81	0.03
0		211	325	131	Not recorded
		151	331	136	Not recorded
		215	277	145	Not recorded
		174	306	120	Not recorded
		248	268	143	Not recorded
	Avg	199.8	301.4	135	
	SD	37.84	28.13	10.07	
	SE	16.92	12.58	4.51	

Appendix 5. Uncorrected Eh mV for sediments comparing "old" and "new" probes in the experiments of Table 5. Reaction temperature = 25°C. Three replicated readings were made for each of the four treatments. Sediments came from the intertidal of Lime Kiln Bay.

Treatment	"Old" probes		"New" probes	
	DW # 1	DW # 2	DW #3	DW #4
4 Molar KCl filling solution				
40 mL sediment - well mixed	-330	-375	-181	-216
	-316	-339	-223	-272
	-321	-330	-221	-229
40 mL sediment with 10 mL 0.3M Na_2S added	-500	-432	-513	-528
	-500	-450	-514	-507
	-500	-453	-510	-507
KNO_3/KCl filling solution				
40 mL sediment - well mixed	-370	-292	-347	-331
	-367	-294	-340	-314
	-378	-311	-354	-319
40 mL sediment with 10 mL 0.3M Na_2S added	-519	-483	-475	-452
	-530	-485	-475	-454
	-521	-486	-467	-448

Appendix 6. Eh mV unconverted for the aqua regia experiment as summarized in Table 6. A is Zobell's A, B is Zobell's B and Sed is a fresh, well-mixed sediment interfacial sample from Pottery Creek, St. Andrews. Four different probes were used as shown.

	"Old" Probes						"New" Probes					
	DW #1			DW # 2			DW #3			DW #4		
Replicate	A	B	Sed	A	B	Sed	A	B	Sed	A	B	Sed
BEFORE PROBE TREATMENT												
1	248	309	48	228	295	-179	236	302	205	246	313	105
2	244	309	58	232	298	-240	235	302	242	242	310	117
3	244	308	50	232	299	-256	235	303	226	241	309	152
4	243	307	8	232	298	-266	234	302	241	240	308	141
5	242	306	-91	231	298	-271	235	302	242	238	306	167
6	240	304	-59	231	298	-285	230	298	241	235	302	198
7	239	303	-75	231	298	-294	230	298	234	235	302	213
8	237	302	-61	231	298	-258	231	299	235	236	303	213
9	237	302	-123	231	298	-268	231	299	241	234	302	207
10	237	301	-106	231	298	-249	231	299	243	233	301	198
Mean	241.1	305.1	-35.1	231	297.8	-256.6	232.8	300.4	235	238	305.6	171.1
SD	3.73	3.07	69.43	1.15	1.03	31.60	2.39	1.96	11.79	4.16	4.20	40.59
SE	1.67	1.37	31.05	0.52	0.46	14.13	1.07	0.87	5.27	1.86	1.88	18.15
AFTER AQUA REGIA PROBE TREATMENT												
1	232	296	-330	236	298	-206	234	299	203	233	300	102
2	235	297	-356	235	298	-115	235	299	226	233	300	68
3	230	296	-351	236	298	-111	235	299	212	233	300	40
4	234	297	-326	236	299	-119	235	299	203	232	300	19
5	235	299	-350	237	299	-116	236	300	238	232	299	-13
6	230	296	-331	237	299	-126	235	300	228	232	299	-15
7	232	298	-371	237	300	-135	234	299	241	234	300	-15
8	233	298	-320	238	300	-117	234	299	237	234	300	-24
9	231	297	-303	237	300	-120	234	299	238	235	301	-30
10	232	295	-367	238	300	-122	234	299	245	235	301	-40
Mean	232.4	296.9	-340.5	236.7	299.1	-128.7	234.6	299.2	227.1	233.3	300	9.2
SD	1.84	1.20	21.88	0.95	0.88	27.96	0.70	0.42	15.78	1.16	0.67	46.95
SE	0.82	0.54	9.79	0.42	0.39	12.50	0.31	0.19	7.06	0.52	0.30	21.00

Appendix 7. Typical data for the SAOB:standard experiment as shown in Fig.4. The volume (5 mL) of standard is kept constant, SAOB volume varied. Before/after standards are with 5 mL each of SAOB:standard taken at the beginning and end of this test. The concentrations are in µM of sulfide, the "adjusted" column is adjusted for dilution involved in adding SAOB.

	Sulfide before		Sulfide after	
Standard µM	µM	mv	µM	mv
10000	10000	-802	10900	-803
1000	1000	-769	1010	-772
100	100	-737	111	-739

	Standard (5 mL), 1000 µM			
SAOB(mL)	mv	$S^{=}$	Dilution	Adjusted
5	-769	1000	1	1000
15	-766	735	2	1470
35	-760	465	4	1860
55	-756	354	6	2124
75	-753	288	8	2304
95	-750	240	10	2400